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Published in:
American Journal of Transplantation

DOI:
10.1111/ajt.14048

Publication date:
2017

American Journal of Transplantation 2017; 17: 1020–1030
Wiley Periodicals Inc.

doi: 10.1111/ajt.14048

Early Conversion to Prednisolone/Everolimus as an Alternative Weaning Regimen Associates With Beneficial Renal Transplant Histology and Function: The Randomized-Controlled MECANO Trial

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Trial registration: NTR1615.

In renal transplantation, use of calcineurin inhibitors (CNIs) is associated with nephrotoxicity and immunosuppression with malignancies and infections. This trial aimed to minimize CNI exposure and total immunosuppression while maintaining efficacy. We performed a randomized controlled, open-label multicenter trial with early cyclosporine A (CsA) elimination. Patients started with basiliximab, prednisolone (P), mycophenolate sodium (MPS), and CsA. At 6 months, immunosuppression was tapered to P/CsA, P/MPS, or P/everolimus (EVL). Primary outcomes were renal fibrosis and inflammation. Secondary outcomes were estimated glomerular filtration rate (eGFR) and incidence of rejection at 24 months. The P/MPS arm was prematurely halted. The trial continued with P/CsA (N = 89) and P/EVL (N = 96). Interstitial fibrosis and inflammation were significantly decreased and the eGFR was significantly higher in the P/EVL arm. Cumulative rejection rates were 13% (P/EVL) and 19% (P/CsA), (p = 0.08). A post hoc analysis of HLA and donor-specific antibodies at 1 year after transplantation revealed no differences. An individualized immunosuppressive strategy of early CNI elimination to dual therapy with everolimus was associated with decreased allograft fibrosis, preserved allograft function, and good efficacy, but also with more serious adverse events and discontinuation. This can be a valuable alternative regimen in patients suffering from CNI toxicity.

Abbreviations: AE, adverse events; AUC, area-under-the concentration-over-time curve; CNI, calcineurin inhibitors; CsA, cyclosporine; eGFR, estimated glomerular filtration rate; EVL, everolimus; IF/TA, interstitial fibrosis/tubular atrophy; MPS, mycophenolate sodium; mTOR, mammalian-target-of-rapamycin; P, prednisolone; PSR, picro sirius red; SAE, serious adverse events

Received 01 March 2016, revised 20 August 2016 and accepted for publication 07 September 2016

Introduction

Renal transplantation is the preferred treatment for patients with end-stage renal failure. The optimal maintenance immunosuppressive therapy after renal transplantation remains to be defined. Premature death by cardiovascular, malignant, and infectious causes is associated with, and in part directly attributable, to the

prolonged use of immunosuppressive drugs and their cumulative load (1). In the last decade, several studies have addressed the optimal strategy to minimize exposure to calcineurin inhibitors (CNIs) (2–4). Apart from their unfavorable cardiovascular risk profile, both CNIs tacrolimus and cyclosporine are inherently nephrotoxic and associated with premature graft loss (5). A recent meta-analysis comprising 11 337 transplant recipients showed that reduction of the CNI improves graft survival (6). On the other hand, it has become evident that under-immunosuppression with ongoing low-grade alloimmunity may also contribute to chronic graft failure (7). Therefore, substitution of a CNI by a nonnephrotoxic mammalian-target-of-rapamycin (mTOR) inhibitor will only preserve graft function when the allo-antibody response is also effectively suppressed. Complete avoidance and replacement of a CNI by everolimus (EVL) in *de novo* transplant recipients is not justified, since this strategy results in unacceptable high acute rejection rates even with induction therapy (8). Both the CAESAR and the SYMPHONY studies show that reduced CNI dosing, as opposed to full-dose CNI, is equally efficacious in preventing acute rejection but only marginally improves renal function (9,10). The CONVERT and the ASCERTAIN studies were initiated to replace CNIs by a mTOR inhibitor late (i.e. 3.2 and 5.6 years, respectively), after transplantation (4,11). This strategy proved to be safe but again only minor improvements in renal function were found, predominantly in patients with still-preserved renal function. In contrast, in the ZEUS study, renal allograft recipients were converted from CNI to EVL at 4–5 months after transplantation to a triple drug regimen with mycophenolate and steroids. This study reported significantly better renal function up to 5 years after CNI elimination with similar graft loss, mortality, and incidence of serious adverse advents (SAEs) (12).

The objective of the present study was to minimize exposure to CNI and the total amount of immunosuppression and to conserve renal allograft function by switching to a non-nephrotoxic double drug regimen early after transplantation, while maintaining efficacy. All patients started with quadruple immunosuppressive therapy with exposure-controlled cyclosporine A (CsA) minimization. At 6 months, patients were assigned to their allocated treatment consisting of dual therapy with steroids and either CsA, mycophenolate sodium (MPS), or EVL. Primary outcome of this study was the development of renal allograft fibrosis 2 years after transplantation. Secondary outcomes were renal function, rejection rates, and adverse events (AEs). We hypothesized that exposure to reduced-dose CsA with mycophenolate and basiliximab induction followed by early CsA elimination and switch to double therapy with everolimus would be associated with less intragraft fibrosis and inflammation and better renal function, without increasing rejection rates. Furthermore, we investigated whether maintenance immunosuppression with EVL as compared to cyclosporine was associated with a rise in donor-specific HLA antibodies.

Methods

Study design and patient population

The study was approved by the local institutional review board. A detailed description of the design of the study and randomization methods have been published previously (13). A 24-month prospective, multicenter, open-label randomized controlled trial was conducted in three university hospitals in the Netherlands. Patients between the ages of 18 and 70 years receiving a first or second renal transplant from a deceased or living donor were eligible. Main exclusion criteria were a HLA-identical sibling donor, a third or fourth transplant, and current or historical panel reactive antibodies of more than 50% and A-B-O incompatibility.

Immunosuppression during the first 6 months after transplantation consisted of two doses of 20 mg of basiliximab intravenously (i.v.), administered prior to transplantation and on day 4, Di-adreson-F 2 × 50 mg i.v. during the first 48 h followed by oral prednisolone (P) 10 mg daily, MPS 2 × 720 mg from the first postoperative day, and CsA. Drug exposure of CsA after transplantation was monitored by serial sampling and calculation of 12-h areas-under-the concentration-over-time curve (AUC_{12}). Target values of AUC_{12} for CsA were 5400 μg*h/L for the first 6 weeks, and thereafter 3250 μg*h/L. Scheduled biopsies were performed at 6 months after transplantation. Patients without rejection in the 6-month scheduled biopsy underwent balanced randomization (1:1:1) to one of the following treatment arms: (1) CsA (target AUC_{12} 3250 μg*h/L), (2) MPA (target AUC_{12} 40 mg*h/L) or a trough level >2 mg/L, or (3) EVL (target AUC_{12} 150 μg*h/L). All patients continued on P 5–10 mg daily.

During the trial, the Data Monitoring Committee reviewed unblinded data and concluded that the P/MPS group had a significantly higher incidence of acute rejection after randomization and consequently this study arm was halted and changes to the protocol were amended (13).

In this investigator-driven trial, there was no independent external monitoring. Monitoring of SAEs relied solely on the assessment of the investigators. The trial was followed by an Independent Safety Monitoring Board monitoring.

Concomitant therapy

Concomitant therapy consisted of a proton pump inhibitor, antihypertensive medicines, and atorvastatin, when needed. Biopsy-proven rejection was treated with methylprednisolone pulses. Refractory rejection episodes were treated with rabbit antithymocyte globulin (Pasteur Merieux, Marnes-la-Coquette, France).

Therapeutic drug monitoring

AUC_{12}s for CsA and EVL were calculated from blood samples drawn at C0, 1, 2, 3, 4, 5, and 6 h after administration. Pharmacokinetic monitoring and clinical assessments were performed at week 2, and months 3, 6, 7, 12, 18, and 24. At week 6 or whenever indicated for clinical reasons, an AUC_{12} was calculated using only three blood samples drawn at 0, 2, and 3 h.

Protocol renal allograft biopsies

Protocol biopsies were scheduled at 6 and 24 months after transplantation. Tissues were formalin-fixed and paraffin-embedded and stained with periodic-acid Schiff diastase, hematoxylin/eosin, and Jones' methenamine silver. Two independent renal pathologists (Leiden University Medical Centre, Leiden and Academic Medical Centre, Amsterdam), unaware of

any clinical data, classified the biopsies according to the latest update of the Banff classification. Interobserver concordance for interstitial inflammation and interstitial fibrosis was good (Kendall W coefficient of concordance 0.68 and 0.80, respectively). The total percentage of inflamed cortical area (ti-score), a continuous score as defined by Mengel et al (14), correlated well between pathologists (Spearman ρ 0.66, $p < 0.0001$). Biopsies that met the minimal adequacy threshold of seven glomeruli and one artery were included for analysis. At 6 months, biopsies were obtained in 99%, 97%, and 98% of patients in the CsA MPS and EVL arm, respectively. Of the available biopsies, 78%, 63%, and 81% in the CsA, MPS, and EVL arm were considered adequate, respectively. At 24 months, biopsies were obtained in 84% and 79% of patients in the CsA and EVL arm, respectively. The prevalence of adequate samples was 81% and 73% in the CsA and EVL arm, respectively ($p = 0.4$, two-tailed).

Morphometric analysis

The morphometric analysis of cortical interstitial fibrosis was centralized at the AMC, Amsterdam. Adequate protocol biopsies were stained for picro sirius red (PSR). PSR-stained slides were digitized using a slide virtual microscope system (Olympus, Tokyo, Japan) using a 20× objective and saved in TIFF format. Vessels that were larger than their adjacent tubuli, glomeruli, the 0.5-mm subcortical area, and the medulla were manually removed. Image analysis was performed with the ImageJ software package (National Institutes of Health, Bethesda, MD). A macro measured the PSR-stained area and the total tubulointerstitial area of the biopsy. All input was manually verified.

Primary and secondary endpoints

The primary endpoints of the study were the development of interstitial fibrosis at the 24-month protocol biopsy (morphometric analysis and Banff interstitial fibrosis/tubular atrophy [IF/TA] score). Secondary endpoints of the study were the estimated glomerular filtration rate (eGFR; estimated with the modification of diet in renal disease algorithm), the incidence of acute rejection, and drug-related AEs (15). Diabetes was defined as the need for antidiabetic drugs. In retrospect, generation of *de novo* HLA class I and II donor-specific antibodies by Luminex assay at 12 months for those patients with available stored serum was additionally determined.

Statistical analysis

Data from the MECANO trial were analyzed as an intention-to-treat estimand. Differences in baseline parameters between the excluded and randomized patients were calculated by independent sample t-tests and chi-square tests where appropriate. In case of graft failure, an eGFR of 10 mL/min and the highest classified inflammation and fibrosis scores were imputed. In the case of death, data were not imputed and were considered missing. The difference in eGFR trajectories was analyzed by a linear mixed-effects model. The cumulative incidence of clinical

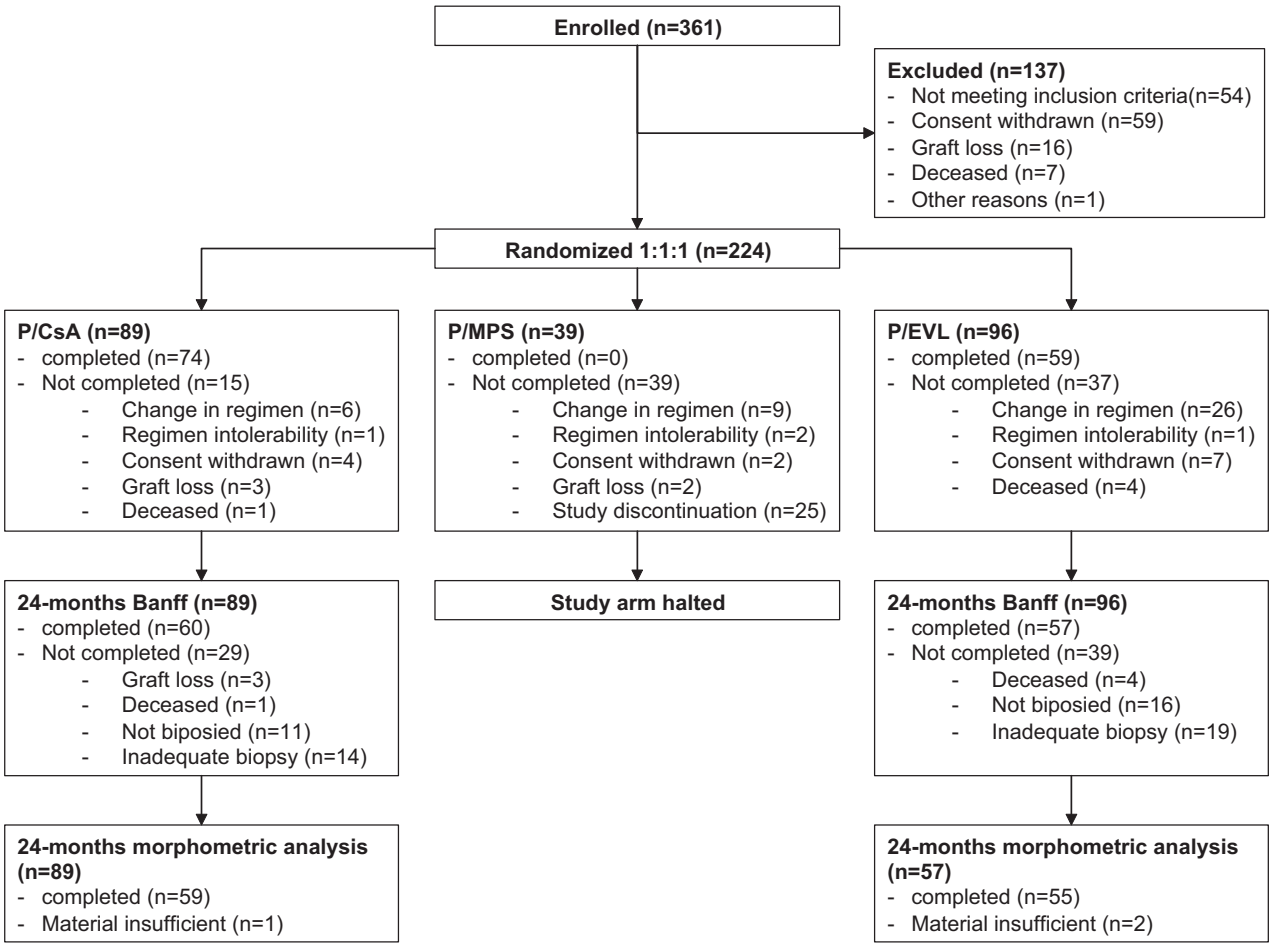

Figure 1: Flow chart of the enrolled patients. P, prednisolone; MPS, mycophenolate sodium; CsA, cyclosporine; EVL, everolimus.

rejection was compared between treatment groups in a time-to-event structure with a log-rank test. Differences in ordinal data (i.e. Banff scores) were calculated with Mann–Whitney ranks tests. Differences in continuous parameters were calculated with use of independent sample t-tests. Differences in *de novo* donor-specific antibodies (DSA) were calculated by chi-square tests. A two-sided p-value of 0.05 was used as a significance threshold. Data were analyzed in the R computational environment version 2.15.2 for Macintosh (www.r-project.org). Prior to the study, the following power analysis was performed based on data of the quantitative morphometric analysis that were acquired in our previous study (16). We showed that 6 months after renal transplantation, the incidence of graft fibrosis was 12%. We assumed that after 24 months the incidence of graft fibrosis in the cyclosporine-treated patients would be 40% and 30% in the two other intervention arms. In order to have a power of 0.80 and an alpha of 0.05, we needed 70 patients per treatment group, taking into account that at 24 months, 85% of patients would reach the primary endpoint. Since there was a high correlation between the co-primary endpoints, (i.e. fibrosis by morphometric analysis and by the Banff score), adjustment of the p-value by for multiple testing was not necessary (17).

Results

Randomization and baseline characteristics

From November 2005 to June 2009, a total of 361 patients were enrolled (Figure 1). Of these patients, 85 ended the study before the 6-month protocol biopsy mainly due to the intensity of the study protocol. Two hundred seventy-five patients underwent a scheduled biopsy. Fifty of these patients showed a Banff type borderline or 1A rejection in this biopsy, whereas in three others Banff class 1b or more was noted. Another three patients were not further tapered to their allocated regimen due to their specific need for an immunosuppressive regimen other than the assigned treatment. In five patients the immunosuppressive therapy was mistakenly tapered. Of the 89 patients assigned to P/CsA, 73 were still on the trial medication at the end of the study versus 58 of 96 patients in the P/EVL group (p = 0.0012, chi-square). On recommendation by the data safety monitoring board, the P/MPS arm was prematurely halted after enrollment of 39 patients due to a significantly higher number of acute rejections (13). The trial continued as a two-arm trial comparing P/EVL with P/CsA. Baseline characteristics of the randomized patients are depicted in Table 1. Table S1 shows the characteristics of the randomized patients compared to the excluded patients. Table S2 shows the primary outcomes in all enrolled patients. Follow-up in groups 1 and 3 was 730 (2–730) and 730 (6–730) days (median [minimum–maximum]).

Therapeutic drug monitoring

Mean CsA $AUC_{12\ hrs}$ at 6 months was 3280 ± 971 μg*h/L. There were no significant differences between patient groups. At 24 months, mean CsA $AUC_{12\ hrs}$ was 3278 ± 907 μg*h/L. Drug exposure to EVL was 203 ± 21 μg*h/L 1 month after conversion and 159 ± 44 μg*h/L at 24 months.

Longitudinal data showing trough levels and daily doses are listed in Table 2.

Table 1: Baseline characteristics of the randomized patients

	P/CsA	P/EVL
Recipient (N)	89	96
Age (years)	49 ± 13	51 ± 13
Male sex (%)	63	65
BMI	25 ± 4	25 ± 3
Race (%)		
European	91	84
Mediterranean	1	6
Asian	5	7
Black	1	2
Other	2	0
Cause of end-stage renal disease (%)		
Glomerulonephritis	19	18
Diabetes mellitus	2	4
Pyelonephritis or interstitial nephritis	3	3
Focal segmental glomerulosclerosis	3	4
Urologic	6	10
Polycystic kidney disease	24	21
Hypertension	15	16
Unknown	5	5
Other	24	19
Donor		
Type of donor (%)		
Post mortal—heart-beating	26	29
Post mortal—non-heart-beating	15	19
Living related	26	22
Living unrelated	34	30
Donor age (years)	49 ± 14	49 ± 13
Donor male sex (%)	53	61
Antigen mismatches—A, B, and DR (no.)	3 ± 2	3 ± 2
Cold-ischemia time—deceased donors only (h)	17 ± 5	16 ± 5
Delayed graft function (%)	12	16
First transplants (%)	96	94

Plus–minus values are means ± standard deviation.
P, prednisolone; CsA, cyclosporine; EVL, everolimus.

Graft fibrosis

Interstitial fibrosis at 24 months as measured by PSR staining was lower in the P/EVL group compared to the P/CsA group (mean difference 6%, 95% CI 3–10%, p = 0.001) (Table 3). $Delta_{6–24}$ interstitial fibrosis percentage was lower in the P/EVL as well (mean difference 6%, 95% CI 1–10%, p = 0.01). The 24-month IF/TA score was lower in the P/EVL group (W = 2143.5, p = 0.03) as was the $delta_{6–24}$ IF/TA score (W = 1465, p = 0.1) (Table 3). There was a trend towards a lower arteriolar hyalinosis and transplant glomerulopathy score at 24 months in the P/EVL versus the P/CsA group (all $p \leq 0.1$, Table 3). The percentage of sclerosed glomeruli and the transplant vasculopathy score did not differ between groups.

Clinical and subclinical rejection

Total inflammation at 24 months as well as its $delta_{6–24}$ value was lower in the P/EVL group compared to P/CsA (mean difference at 24 months 14% [95% CI

Table 2: Daily immunosuppressive doses, trough levels, and C2 levels (CsA)

	P/CsA (N = 89)		P/EVL (N = 96)	
	Mean ± SD	Median (min–max)	Mean ± SD	Median (min–max)
Month 6	(N = 89)		(N = 96)	
CsA daily dose (mg)	229 ± 58	200 (100–400)	222 ± 57	200 (100–400)
CsA Ctrough (μg/L)	122 ± 51	110 (33–333)	117 ± 41	113 (25–219)
MPS daily dose	1395 ± 171	1440 (720–1440)	1395 ± 176	1440 (720–1440)
MPA Ctrough (mg/L)	3 ± 3.5	2.1 (0.6–28.5)	3.2 ± 3.6	2.3 (0.5–19.5)
P dose	10 ± 1.0	10 (8–10)	10 ± 1.0	10 (8–10)
Month 7	(N = 89)		(N = 96)	
CsA daily dose	226 ± 32	200 (150–350)		
CsA Ctrough (μg/L)	132 ± 61	119 (30–453)		
CsA C2 (μg/L)	653 ± 207	654 (260–1163)		
EVL daily dose			5.92 ± 0.4	6 (3–9)
EVL Ctrough (μg/L)			10.7 ± 5.8	9.1 (4.2–32.2)
P dose	10 ± 1.0	10 (8–15)	10 ± 0.8	10 (5–10)
Month 12	(N = 77)		(N = 81)	
CsA daily dose	224 ± 54	200 (100–350)		
CsA Ctrough (μg/L)	121 ± 46	112 (33–300)		
CsA C2 (μg/L)	653 ± 207	654 (260–1163)		
EVL daily dose			4.8 ± 0.8	4.5 (2–10.5)
EVL Ctrough (μg/L)			9.3 ± 3.6	8.7 (3.8–23.2)
P dose	10 ± 2.0	10 (5–25)	10.5 ± 5	10 (8–50)
Month 24	(N = 70)		(N = 59)	
CsA daily dose	218 ± 58	200 (100–400)		
CsA Ctrough (μg/L)	123 ± 72	108 (17–469)		
CsA C2 (μg/L)	656 ± 224	628 (130–1389)		
EVL daily dose			4.2 ± 0.7	4.5 (1.5–7.5)
EVL Ctrough (μg/L)			8.9 ± 3.1	8.1 (3.3–16.2)
P dose	10 ± 1.0	10 (8–10)	10 ± 1	10 (5–10)

CsA, cyclosporine; MPS, mycophenolate sodium; MPA, mycophenolic acid; P, prednisolone; EVL, everolimus; SD, standard deviation.

3–24%] and $delta_{6–24}$ 13% [95% CI 3–23%], both p = 0.01, Table 3). At the 24-month protocol biopsy, the prevalence of subclinical rejection-free patients did not differ between the treatment arms (83% vs. 82%, respectively). Banff g, t, and i scores were not significantly different between groups (Table 3). Arteritis was not present in any of the protocol biopsies. After randomization, at 24 months the cumulative incidence of acute clinical rejection in the P/CsA group was 9% as compared to 3% in the P/EVL group (Figure 2A, p = 0.08).

Generation of de novo *donor-specific antibodies after randomization*

Of the patients who finalized the study protocol, 26/69 (38%) patients in the P/CsA and 16/54 (30%) in the P/EVL group developed *de novo* anti-HLA class I or II antibodies (p = 0.35), of which 12/69 (17%) and 5/54 (10%) were DSA, respectively (p = 0.195). No significant differences in *de novo* class I and II DSA were observed (p = 0.12 for class I and p = 0.81 for class II).

Patient survival, graft survival, and graft function

After randomization, overall graft survival was 96% in the P/CsA as well as P/EVL group. Patient survival in the P/CsA group was 99% and in the P/EVL group 96%. In the P/CsA group, death-censored graft survival was 97% compared to 100% in the P/EVL group. The eGFR trajectory in the P/EVL arm was higher at each time-point after randomization as compared to the eGFR trajectory in the P/CsA arm (Figure 2B, p < 0.05, linear mixed-effects model).

Proteinuria and total cholesterol levels at months 6 and 24

Proteinuria (g/24 h) at months 6 and 24 after transplantation was 0.24 (interquartile range [IQR] 0.15–0.36) and 0.20 (IQR 0.13–0.30) in the P/CsA group and 0.22 (IQR 0.14–0.30) and 0.30 (IQR 0.18–0.49) in the P/EVL group (median [IQR]). This was not significant (p = 0.21) (Mann–Whitney rank test).

Cholesterol levels at months 6 and 24 after transplantation are shown in Table 4.

Adverse events

AEs and SAEs are depicted in Table 5. There were significantly more SAEs in the P/EVL group as compared to the P/CsA group after effective allocation to treatment (p < 0.001, chi-square). In the P/CsA group, one patient died due to myocardial infarction. In the P/EVL group, four patients died: two by "sudden death," one after a stroke,

Table 3: Quantitative histological analysis of the protocol biopsies according to the Banff 2013 classification

	P/CsA	P/EVL	p-value
Graft inflammation			
t-score			
At 6 months	0 (0–0), 21%	0 (0–0), 14%	
At 24 months	0 (0–1), 35%	0 (0–1), 32%	0.6[1]
$Delta_{6–24}$	0 (0–0.5), 25%	0 (0–0.75), 26%	0.9[1]
i-score			
At 6 months	1 (0–1), 57%	1 (0–1), 57%	
At 24 months	1 (0–1.5), 71%	1 (0–2), 66%	0.7[1]
$Delta_{6–24}$	0 (0–1), 40%	0 (0–1), 35%	0.8[1]
g-score			
At 6 months	0 (0–0), 1%	0 (0–0), 3%	
At 24 months	0 (0–0), 16%	0 (0–0), 7%	0.1[1]
$Delta_{6–24}$	0 (0–0), 13%	0 (0–0), 7%	0.3[1]
ti-score, %			
At 6 months	11 ± 8	13 ± 11	
At 24 months	37 ± 33	24 ± 23	0.01[2]
$Delta_{6–24}$	24 ± 30	11 ± 20	0.01[2]
Graft fibrosis			
IF/TA-score			
At 6 months	1 (0–1), 69%	1 (0–1), 66%	
At 24 months[3]	1 (1–2), 92%	1 (1–2), 88%	0.03[1]
$Delta_{6–24}$	1 (0–1), 56%	0 (0–1), 43%	0.1[1]
cg-score			
At 6 months	0 (0–0), 1%	0 (0–0), 1%	
At 24 months	0 (0–0), 8%	0 (0–0), 2%	0.1[1]
$Delta_{6–24}$	0 (0–0), 5%	0 (0–0), 2%	0.2[1]
cv-score			
At 6 months	0 (0–1), 31%	0 (0–1), 33%	
At 24 months	0 (0–1), 46%	0 (0–1), 45%	>0.9[1]
$Delta_{6–24}$	0 (0–1), 36%	0 (0–1), 43%	0.6[1]
ah-score			
At 6 months	0 (0–0), 24%	0 (0–1), 37%	
At 24 months	0 (0–1), 46%	0 (0–1), 36%	0.1[1]
$Delta_{6–24}$	0 (0–1), 29%	0 (0–0), 24%	0.3[1]
GGS, %			
At 6 months	3 ± 5	3 ± 5	
At 24 months	9 ± 12	6 ± 8	0.2[2]
$Delta_{6–24}$	5 ± 13	3 ± 11	0.5[2]
IF, %			
At 6 months	13 ± 6	14 ± 6	
At 24 months	21 ± 12	15 ± 8	0.001[2]
$Delta_{6–24}$	7 ± 13	1 ± 8	0.01[2]

Ordinal Banff scores are depicted as median (interquartile range), percentage of patients with a score >0. The ti-score, the percentage of GGS, and the percentage of IF are shown as mean ± standard deviation. The percentage represents the fraction of the cortex with fibrosis. Banff v-score values were 0 in all protocol biopsies and therefore not shown.

GGS, global glomerulosclerosis; IF, interstitial fibrosis (morphometric analysis of picro-sirius red staining); TA, tubular atrophy.

[1]Two-sided Mann–Whitney rank test.

[2]Two-sided t-test.

[3]The percentage of patients with IF/TA score >1 at 24 months was 46% in the P/CsA group versus 29% in the P/EVL group.

and one due to carcinoma of the lung. Twenty-six patients in the EVL arm changed their immunosuppressive maintenance regimen. Reasons to switch to a different immunosuppressive regimen other than P/EVL were rejection (3), pneumonitis (10), severe rash (4), edema (2), diarrhea (2), severe malaise (2), pulmonary embolism (1), severe hypertriglyceridemia (1), and BK nephropathy (1).

Discussion

This study is the first prospective study with early complete CsA withdrawal and conversion to dual therapy consisting of prednisolone and everolimus with as primary outcome a quantitative histopathological analysis of fibrosis in scheduled renal transplant biopsies. This morphometric outcome is a good surrogate marker for long-term allograft function and eGFR (18). The study has two other distinct features: (1) prior to effective allocation to the various weaning regimens, scheduled biopsies were analyzed to rule out subclinical rejection, and (2) immunosuppressive drug monitoring was tightly controlled using AUCs instead of trough levels. This study shows that controlled reduced CsA exposure with mycophenolate followed by early CsA withdrawal and dual maintenance therapy with steroids and everolimus is associated with less fibrosis and decreased inflammation as compared to dual therapy with steroids and CsA. Both chronic histological damage and the total inflammation score are important predictors of renal allograft outcome (14,19).

Histological lesions such as interstitial fibrosis, tubular atrophy, and arteriolar hyalinosis are associated with CNI toxicity but also occur in renal allograft recipients not on CNIs (20). Enhanced allo-immunity is assumed to be a second important risk factor for the development of these lesions (21). In this study, everolimus proved to be very efficacious, and significantly better than MPS, to suppress the allo-response, provided that, prior to withdrawal, drug levels were adequate and subclinical rejection was excluded. The previously reported differences in efficacy between CNIs and mTOR inhibitors (8) are in contrast with our study, but can be explained by the controlled drug exposure and/or the absence of drug–drug interactions (22). In the present trial, exposure to CsA and EVL were controlled using AUC12 h instead of trough levels. CsA trough levels especially have been shown to correlate poorly with systemic exposure and clinical outcome parameters (23). Our data are in concordance with those of Chhabra et al, who demonstrated in a randomized single-center study that tacrolimus replacement by sirolimus in a dual maintenance regimen with MPA was equally efficacious as the control arm that continued on tacrolimus. However, in the latter study no beneficial effect on either graft histology or on clinical parameters was reported, possibly due to the late time point of conversion (24). A second explanation of the better histological outcome in the everolimus arm may be intrinsic antifibrotic properties ascribed to mTOR inhibitors (25).

The histological data of the study are in line with the renal allograft function. The mean eGFR at 2 years after

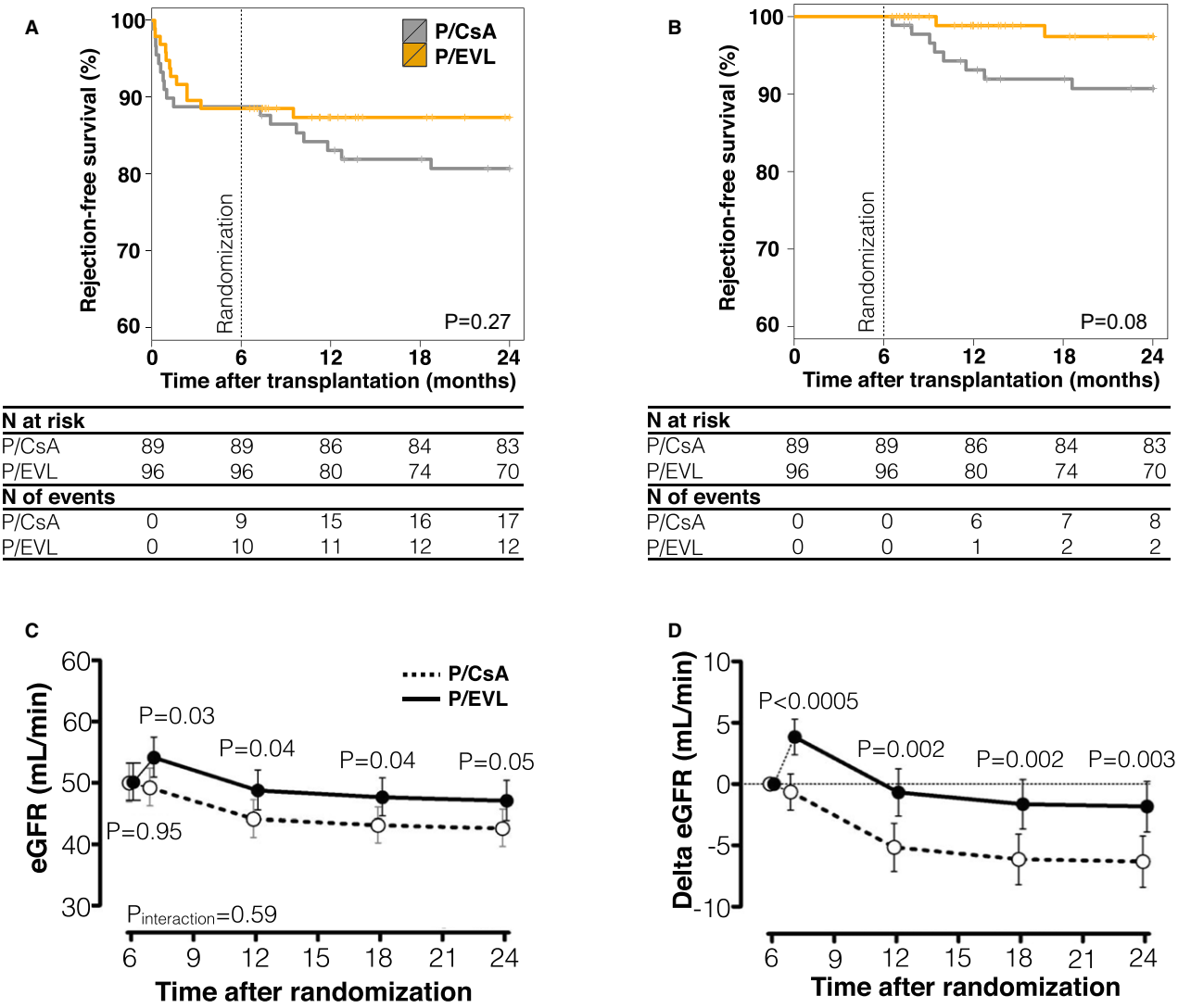

Figure 2: Clinical renal outcome from the P/EVL and P/CsA groups. Rejection-free survival from t = 0 (A) and t = 6 months (B) and eGFR trajectories (C) and delta eGFR trajectories (D). P, prednisolone; EVL, everolimus; CsA, cyclosporine; eGFR, estimated glomerular filtration rate.

Table 4: Cholesterol and proteinuria levels at month 6 and month 24

		P/CsA (n = 89)			P/EVL (n = 96)			
	Time	N	Mean	SD	N	Mean	SD	p
Cholesterol (mmol/L)	Month 6	87	5.22	1.04	96	5.18	1.01	n/a
	Month 24	80	5.12	0.98	89	5.40	1.13	0.088[1]
Proteinuria g/24 h	Month 6	86	0.23	0.15–0.36	95	0.22	0.14–0.30	n/a
	Month 24	82	0.20	0.13–0.30	88	0.30	0.18–0.48	0.209[2]
Proteinuria >0.5 (g/24 h)	Month 24	12/82	14.6%		19/88	21.6%		0.240[3]
Statin use	Month 6	52/87	59.8%		54/96	56.3%		n/a
	Month 24	53/77	68.8%		51/72	70.8%		0.239[3]

Proteinuria g/24 h are presented as median (IQR).
P, prednisolone; CsA, cyclosporine; EVL, everolimus; SD, standard deviation; n/a, not applicable.
[1]Tested with independent samples t-test.
[2]Tested with Mann–Whitney rank test.
[3]Tested with Pearson chi-square.

Table 5: Confirmed adverse events (AE) and serious adverse events (SAE)

	Before randomization 0–6 months				After randomization 6–24 months			
	AE		SAE		AE		SAE	
	P/CsA	P/EVL	P/CsA	P/EVL	P/CsA	P/EVL	P/CsA	P/EVL
Total episodes	59	77	26	22	40	61	32	71
Total patients with episode	35 (39%)	44 (46%)	18 (20%)	12 (13%)	36 (40%)	43 (45%)	19 (21%)	44 (46%)
	p = 0.12		p = 0.12		p = 0.68		p < 0.01	
Gastrointestinal—total	0	2	2	1	2	2	4	7
Gingiva hyperplasia	0	0	0	0	1	0	0	0
Diarrhea	0	2	1	1	1	2	3	7
Other gastrointestinal event	0	0	1	0	0	0	1	
Urinary tract complications—total	35	26	12	8	14	19	6	12
Urinary tract infection	33	26	4	2	6	18	4	4
Urosepsis	1	0	5	4	5	0	0	8
Urological other	1	0	3	2	3	1	2	0
Respiratory—total	0	2	2	2	0	5	2	11
Pneumonia								
Bacterial	0	2	2	0	0	3	2	0
PCP	0	0	0	2	0	0	0	2
Probably medication—related	0	0	0	0	0	2	0	9
Other infections—total	15	29	6	3	12	9	7	5
CMV								
Primo	0	3	2	0	0	0	2	0
Reactivation	7	15	1	1	7	0	1	1
Herpes zoster	2	1	0	0	2	1	0	0
Herpes labialis	0	3	0	0	0	1	0	0
Infection other	6	7	3	2	3	7	4	4
Malignancies—total	0	0	1	1	0	0	3	3
Skin	0	0	0	0	0	0	2	1
Nonskin	0	0	1	1	0	0	1	2
Cardiovascular—total	4	3	1	3	2	2	5	12
Infarction	1	0	0	0	0	0	1	1
Venous thrombosis	1	1	1	1	0	1	0	7
Lung embolus	0	0	0	0	0	0	0	2
Peripheral arterial disease	1	0	0	0	0	0	1	1
Other cardiovascular event	1	2	0	2	2	1	3	1
New-onset diabetes mellitus	2	3	0	0	4	14	0	0
Gout	1	0	0	0	1	0	0	0
Flulike symptoms	1	2	0	0	1	0	5	0
Edema	0	4	1	0	1	2	0	0
Other	1	6	1	4	3	8	0	21
Death	n/a	n/a	n/a	n/a	0	0	1	4

SAE < 6 months: P/CSA gastrointestinal other: abdominal ileus, urological other: urethrotomy, acute urinary retention, hydronephrosis. Infection other: peritoneal catheter–related peritonitis (2), bacteremia with *Staphylococcus aureus*, Other transient rise in creatinine. P/EVL urological other; Hydronephrosis, replacement of by Boari splint, Infection other; fever of unknown cause, *Escherichia coli* bacteremia, Other: ablation of the retina, shortness of breath, acute tubulus necrosis, cognitive impairment. AE <6 months. P/CsA Urological other; hydronephrosis, Infection other: perianal abscess (2), culture-positive preservation fluid, wound infection, peritonitis, infected liver cyst. Other; tendinitis P/EVL Pos PCR EBV (2), wound infection, BK virus nephropathy, exacerbation chronic hepatitis B, infected preservation fluid, oral candida Other; drug hepatitis, leucopenia, acute tubulus necrosis (3), rhinitis. SAE > 6 months P/CsA Other Urological urethra stricture, lymphocele, Infection other infected renal cyst, abdominal wall, abscess (2), fever of unknown origin, Other cardiovascular; femoral bypass, subdural hematoma, arterial percutaneous angioplasty P/EVL Other Infection; fever of unknown origin, BK nephropathy, infected renal cyst, colitis, Cardiovascular Other: angioplasty of shunt. Other: Eyelid correction, cataract (3), ablation of the retina, shunt problems (2), pain thorax wall, melena, angioedema, rise in creatinine (2), malaise (2), stenosis in the spine, nephrectomy native kidney, skin rash (4), hypertriglyceridemia. AE >6 months P/CsA Other infection otitis media (2), upper respiratory infection. Cardiovascular Other; shunt thrombosis, Other arthrosis, gingiva hyperplasia, bleeding from the gastrointestinal tract, vaginal bleeding P/EVL Urological other; dysuria, Infection other; candida infection (3), bronchitis (2), abscess groin, fever of unknown origin, Other; ulcers in mouth, shortness of breath (2), dyspepsia, skin rash, malaise, fracture tibia, luxation of the scapula.
P, prednisolone; CsA, cyclosporine; EVL, everolimus; PCP, *Pneumocystis* pneumonia; CMV, cytomegalovirus; PCR, polymerase chain reaction; EBV, Epstein–Barr virus; n/a, not applicable.

transplantation in the current study was 7 mL/min higher in the EVL group as compared to patients in the CsA group. The patients on CsA have a slow but progressive decline in their eGFR, which is not observed in the EVL arm.

We included patients with a standard-to-intermediate immunological risk profile. The overall HLA matching rate is relatively poor due to high numbers of living unrelated donors. Protocol biopsies at 6 months were performed to reduce the immunological risk, since patients with inflammation in their surveillance biopsies did not proceed to their assigned dual treatment arm. After tapering the triple regimen to double therapy, the 2-year cumulative rejection incidences were 9% in the P/CsA group and only 3% in the P/EVL group. In contrast, in the ZEUS study patients converted to EVL experienced a higher rejection rate as compared to those who continued on triple therapy with cyclosporine. The difference may be explained by the approach in therapeutic drug monitoring or due to the fact that in the ZEUS study no preconversion biopsies were performed to exclude subclinical rejection. The results in the current study are more striking because in the ZEUS study, CsA elimination with EVL was performed in the presence of mycophenolate.

We performed a post hoc analysis of the *de novo* incidence of HLA antibodies and DSAs at 1 year after transplantation. In a single-center study, the CNI-free regimen was associated with an increased incidence of DSAs (26). However, in this study a large part of the patients did not use prednisolone, which might have resulted in underimmunosuppression. We and others did not find such an increase in DSAs in the EVL-treated patients (27).

Tolerability was significantly lower in the P/EVL group. At 2 years, only 60% of the patients were still on the assigned treatment as compared to 88% of the P/CsA patients. This is compatible with data from the literature (28). Like others, we did not find a significant correlation between the level of EVL exposure and side effects.

We did not find a difference between cardiovascular events between the two groups nor in cholesterol levels. In our analysis, cholesterol levels at 24 months were not significantly increased as compared to levels at effective randomization or between the two groups. However, most patients needed statins to control their cholesterol levels. EVL is associated with a dose-dependent increased risk of hypercholesterolemia (29). This has raised concern about the effect on cardiovascular events. A recent observational cohort study comprising 9353 adult kidney transplant recipients with a median follow-up of 7 years showed a higher risk of all-cause mortality with mTOR use, as compared to CNI use. This risk, however, was largely explained by the increased risk of death by malignancy, suggestive of an indication bias. In this study, death by cardiovascular causes was not independently increased; however, results should be interpreted with caution since numbers in this subgroup analysis were small (30).

On the other hand, mTOR inhibitors possibly have cardioprotective effects; in a recent trial in 721 *de novo* heart transplant patients randomized either to EVL and reduced-dose CsA or CsA and mycophenolate mofetil, a subanalysis of 185 patients showed significantly less intimal thickness of the coronary arteries in the EVL-treated patients. This was independent of cholesterol levels (31).

There are several limitations to the current study that should be considered. First, we used CsA as the comparator drug and not tacrolimus. Whether the eGFR in a P/tacrolimus arm would have been better is speculative. In the 3-year follow-up of the SYMPHONY study, the eGFR was only slightly better in the tacrolimus arm as compared to the standard-dose CsA arm, but not in comparison to the reduced-dose CsA (24). Secondly, in our study a comparator arm with a triple-drug calcineurin-based immunosuppressive regimen is lacking. After MPS withdrawal, an additional 9% of the CsA experienced an acute rejection. However, survival without (borderline) rejection for the CsA and EVL treatment arms was 83% and 82%, respectively. For this selected group of patients, with no signs of subclinical rejection in the 6-month scheduled biopsy, the borderline-free rejection survival was similar to the 1-year outcome of the best arm in the Symphony trial. In this trial, borderline-free rejection survival was 85% in the Low-Tac arm (32). Thirdly, most of our patients were from a white background and, although based on controlled systemic exposure, we cannot extrapolate our findings to other ethnic groups. Fourthly, our AUC of EVL were relatively high (target AUC12 150 μg*h/L), which is compatible with target trough levels of 8–12 μg/L. Finally, our follow-up was only 2 years.

In conclusion, this trial included *de novo* renal transplant recipients with a standard-to-intermediate immunological risk. The results show that after exclusion of subclinical rejection at 6 months, quadruple therapy followed by CsA elimination with EVL in dual therapy with prednisolone is safe, slows the progression of interstitial fibrosis and inflammation, and preserves renal allograft function. Furthermore, there were no differences between the groups in the incidence of *de novo* HLA antibodies.

In a low-immunological risk group of patients, double therapy with prednisolone and EVL can be a good alternative to a CNI-containing regimen.

Acknowledgments

Without the effort of all participating patients this study would not have been possible. In addition, we thank S. Hendriksen, M. van Dijk, G.

Nieuwenhuizen, N. Claessen, and O.J. de Boer for their excellent help during the study. This trial was an investigator-originated, -initiated, and -driven trial performed by three university hospitals in the Netherlands. Novartis Pharma financially supported the execution of the study by an unrestricted grant. The authors performed data collection, statistical analysis, and writing of the manuscript. All authors shared the final responsibility for the decision to submit the manuscript for publication.

Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1: Baseline characteristics of the enrolled patients.

Table S2: Primary clinical outcome at 6 months.